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FINAL REPORT

BIOLOGICAL SPECIMEN STORAGE FOR EXTENDED SPACE MISSIONS

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FOREWORD

This study was sponsored by the National Aeronautics and Space Administration under Contract Number NASW-1556. Portions of the study effort were conducted under subcontract to Bio-Science Laboratories, Inc. A companion report entitled Physical Methods for Biochemical Analysis in Spaceflight is available from the contractor as Report Number SR67-1044.

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INTRODUCTION

A complete evaluation of the effects of long-term weightlessness and confinement on man as experienced during an extended space mission requires a number of biochemical tests similar to those performed for the physician by a clinical laboratory. These tests may be accomplished in two ways: in-flight as part of the mission, or post-flight, on specimens obtained during the flight and stored. The storage problem is unique because of the duration anticipated (up to 90 days) and because of the limitations imposed by flight conditions.

The preservation of biological samples presents a minor problem in most clinical laboratories due to the short time interval between sample collection and actual analysis. When delays are anticipated, it is customary to refrigerate the samples. Longer storage times, however, introduce unique preservation problems, not normally encountered in the laboratory environment.

The purpose of this study is to critically evaluate a number of methods for preserving biological specimens, to determine the feasibility of using the methods in flight, and to recommend a method or combination of methods for preserving and storing specimens collected during space flight for post-flight analysis. Table 1 presents a list of the clinical laboratory evaluations under consideration for the Apollo Applications Program. These parameters are the ones considered for preservation and storage in this study.

Preservation methods considered include:

- Chemical
- Refrigeration
- Freezing

TABLE 1. - LIST OF PARAMETERS AND SPECIMENS TO BE CONSIDERED

Parameter	Serum or plasma	Urine	Whole blood	Feces	Sweat
Adrenocorticotrophic hormone (ACTH)	X				
Aldosterone		X			
Alkaline phosphatase	X				
Amino nitrogen	X				
Antidiuretic hormone (ADH)	X	X			
Antihemophylic globulin (AHG)	X				
Bicarbonate	X				
Bilirubin	X				
BUN	X				
Calcium	X	X		X	X
Caryotyping			X		
Catecholamines		X			
Chlorides	X	X		X	X
Clot retraction			X		
Clotting time			X		
Creatine	X	X			
Creatinine	X	X			
Fat tolerance	X				
Fibrinogen	X				
Fibrinolytic activity	X				
Glucose tolerance			X		
Hematocrit			X		
Hemoglobin			X		
17-Hydroxycorticosteroids		X			
Immune bodies	X				
Lactic acid			X		
LDH isozymes	X				
Magnesium	X	X			
Manganese	X	X			
Methemoglobin			X		
Mucoproteins & related biocolloids	X	X			
NPN	X				
PBI (protein bound iodine)	X				
Phosphates	X				
Plasma thromboplastic component (PTC)	X				

TABLE 1. - LIST OF PARAMETERS AND SPECIMENS TO BE CONSIDERED - Concluded

Parameter	Serum or plasma	Urine	Whole blood	Feces	Sweat
Plasma volume ($RISA^{125}$)	X				
Platelet adhesiveness			X		
Platelet count			X		
Potassium	X	X		X	X
Proteins (electrophoresis)	X				
Prothrombin activity	X				
RBC cell mass (isotopes)			X		
RBC survival			X		
RBC (total)			X		
Reticulocyte count			X		
Serotonin (5-HIAA)		X			
Sodium	X	X		X	X
Standard clinical analysis[1]		X			
Sulfates	X	X			
Thyroxine	X				
Thyroxine binding prealbumin (TBPA)	X				
Total nitrogen		X			
Transferrins	X				
Uric acid	X				
WBC differential			X		
WBC motility and phagocytic activity			X		
WBC (total)			X		
Zinc	X	X			
Microbiological parameters[2]					

[1] Standard clinical analysis includes: volume, color, turbidity, specific gravity, pH, albumin, sugar, ketone bodies, microscopy.

[2] Microbiological parameters include:

Sampling and Culturing of Flora Body
Colony Counts
Microbiological Identifications

The sources for these parameters are not indicated since they were not specified by NASA.

- Vacuum distillation
- Absorption and ion exchange
- Lyophilization

Special biological problems such as hemolysis, protein precipitation, contaminant microbial growths, and the use of anticoagulants, are considered and evaluated. Recommendations and rationale for specimen preservation and storage are included.

SPECIMEN PRESERVATION TECHNIQUES

General

A major consideration in the storage of biological samples is the maintenance of the stability of the specimen during the interval between the time of collection and the time analysis is begun. Many organic substances are subject to deterioration due to bacterial contamination or to the action of enzymes present in situ. The effects of microbial growth are largely unpredictable and become a problem when the sample is to be stored for longer than one day either at room or refrigerator temperature. This problem can be solved through a sterile environment for the collection and storage of all biological material. However, it is usually more practical to freeze the sample or to employ an antibacterial agent. Acidification of the sample may also be employed to retard microbial growth. The degradation of organic material resulting from enzyme action may be blocked by addition of a specific enzyme inhibitor. Alternatively, the sample may be adjusted to a pH value at which the enzyme is no longer active.

Table 1 lists a number of elements and electrolytes which have high inherent stability. These inorganic substances include bicarbonate, calcium, chloride, magnesium, manganese, potassium, sodium, and zinc. In practice,

storage of biological samples containing inorganic substances at room temperature or even under refrigeration for several days leads to a precipitation of protein or inorganic material, or to a growth of microbial contaminants. If the supernatant is later sampled without regard to the debris present in these heterogeneous specimens, serious errors may result in the analysis.

Specimen Collection

Urine collections may be made by methods discussed in Lockheed Report M-61-64-1-I. Sweat is absorbed in gauze as described by Henry and may be stored in plastic containers. When small amounts of blood are required the sample may be obtained by fingertip puncture and drawn into capillary tubes for subsequent analysis. Where larger amounts of blood or plasma are required, the specimen normally is obtained by venipuncture and the blood is discharged into tubes containing an anticoagulant. For serum specimens the anticoagulant is omitted from the collection bottle. Feces may be collected by methods previously developed for NASA.

Many of the parameters normally found in whole blood are present in unequal concentrations in the erythrocytes and in the extracellular plasma phase. In those instances when the parameter is present primarily in the erythrocytes, it is necessary that the analysis be performed on whole blood. Whole blood is required for analysis of hemoglobin and methemoglobin, and for many of the hematological studies. Plasma is specified for blood volume determination, amino nitrogen, and fibrinogen, as well as for studies in clotting dynamics. For most biochemical tests, the specimen of choice is serum, whose preparation does not require the use of an anticoagulant.

It may be noted that the preparation of serum and plasma requires their separation from blood corpuscles within a relatively short period of time after

the blood is obtained to prevent secondary changes in the specimen. For this purpose, a spaceborne laboratory centrifuge or its equivalent must be considered.

The relative merits of various methods of sample preservation are evaluated below for each type of specimen listed in Table 1. Each topical heading is supported by data in the related table. The periods of stability given in these tables generally correspond to a loss of less than 3% in each parameter. The sample quantities required for each analysis are listed in Table 2. This table may be employed as a basis for determining minimum sample quantities when all parameters are to be analyzed in a given specimen.

Notes and abbreviations applicable to Tables 3 through 7 are contained in Appendix A.

Urine

Untreated urine stored at room temperature is unstable for a significant number of the 18 designated parameters. As indicated in Table 3, changes occur in creatine, sugar, ketone bodies and pH within several hours after the urine collection is made. Catecholamines and aldosterone are stable for one to two days at room temperature in the absence of preservatives. Most of the remaining parameters tend to be more stable. Inorganic constituents, such as calcium, chlorides, magnesium, manganese, potassium, sodium, and zinc are stable for long periods of time, but as noted previously, there is a tendency for these substances to precipitate or co-precipitate during standing. It is therefore necessary that the urine specimens be acidified to dissolve these deposits prior to analysis.

A number of chemical agents have been used to preserve urine specimens at room temperature. Among these agents are formaldehyde, toluene, chloroform,

TABLE 2. - APPROXIMATE MINIMUM SAMPLES REQUIRED FOR ANALYSIS IN SINGLICATE

Parameter	Serum or plasma ml.	Urine ml.	Whole blood ml.	Feces g.	Sweat ml.
Adrenocorticotrophic hormone (ACTH)	4.0	---	---	---	---
Aldosterone	---	10.0	---	---	---
Alkaline phosphatase	0.10	---	---	---	---
Amino nitrogen	0.50	---	---	---	---
Antidiuretic hormone (ADH)	1.0	?	---	---	---
Antihemophylic globulin (AHG)	0.20	---	---	---	---
Bicarbonate	0.025	---	---	---	---
Bilirubin	0.10	---	---	---	---
BUN	0.05	---	---	---	---
Calcium	0.10	0.05	---	1.0	1.0
Caryotyping	---	---	0.10	---	---
Catecholamines	2.0	5.0	---	---	---
Chlorides	0.05	0.05	---	1.0	1.0
Clot retraction	---	---	0.50	---	---
Clotting time	---	---	0.50	---	---
Creatine	1.0	0.10	---	---	---
Creatinine	0.20	0.05	---	---	---
Fat tolerance	0.25	---	---	---	---
Fibrinogen	0.50	---	---	---	---
Fibrinolytic activity	0.50	---	---	---	---
Glucose tolerance	---	---	0.25	---	---
Hematocrit	---	---	0.10	---	---
Hemoglobin	---	---	0.05	---	---
17-Hydroxycorticosteroids	---	10.0	---	---	---
Immune bodies	0.10	---	---	---	---
Lactic acid	---	---	0.25	---	---
LDH isozymes	0.10	---	---	---	---
Magnesium	0.20	0.50	---	---	---
Manganese	8.00	?	---	---	---
Methemoglobin	---	---	0.20	---	---
Mucoproteins & related biocolloids	0.50	?	---	---	---
NPN	0.10	---	---	---	---
PBI (protein bound iodine)	0.20	---	---	---	---
Phosphates	0.025	---	---	---	---

NOTE: Values are estimates based on current usage. Some sample requirements may be reduced up to 50% by employing ultramicro analysis techniques, or by performing two or more analyses on the same specimen.

TABLE 2. - APPROXIMATE MINIMUM SAMPLES REQUIRED FOR ANALYSIS IN SINGLICATE - Concluded

Parameter	Serum or plasma ml.	Urine ml.	Whole blood ml.	Feces g.	Sweat ml.
Plasma thromboplastic component (PTC)	0.20	---	---	---	---
Plasma volume ($RISA^{125}$)	2.0	---	---	---	---
Platelet adhesiveness	---	---	0.10	---	---
Platelet count	---	---	0.05	---	---
Potassium	0.05	1.0	---	1.0	1.0
Proteins (electrophoresis)	0.05	---	---	---	---
Prothrombin activity	0.50	---	---	---	---
RBC cell mass (isotopes)	---	---	2.0	---	---
RBC survival	---	---	2.0	---	---
RBC (total)	---	---	0.05	---	---
Reticulocyte count	---	---	0.05	---	---
Serotonin (5-HIAA)	---	2.0	---	---	---
Sodium	0.05	1.0	---	1.0	1.0
Standard clinical analysis	---	1.0	---	---	---
Sulfates	1.0	0.50	---	---	---
Thyroxine	1.0	---	---	---	---
Thyroxine binding prealbumin (TBPA)	1.0	---	---	---	---
Total nitrogen	---	0.20	---	---	---
Transferrins	0.10	---	---	---	---
Uric acid	0.10	---	---	---	---
WBC differential	---	---	0.05	---	---
WBC motility and phagocytic activity	---	---	0.05	---	---
WBC (total)	---	---	0.05	---	---
Zinc	2.00	5.0	---	---	---

NOTE: Values are estimates based on current usage. Some sample requirements may be reduced up to 50% by employing ultramicro analysis techniques, or by performing two or more analyses on the same specimen.

TABLE 3.- DURATION OF STABILITY OF URINARY CONSTITUENTS

Parameter	Method of storage					
	Without preservative	Chemical		Refrigeration	Freezing	Lyophilization
		Preservative	Duration			
Aldosterone	24 hrs[B]	H_3BO_3 (pH 4-5)	4 days[B]	≥ 2 wks (H_3BO_3)[B]	1 yr (H_3BO_3)[B]	
Antidiuretic hormone	sev. days[L]	acid (pH 2.5-4)	1 week[L]	3-4 wks[L]	3-6 mos[L]	
Calcium[1]	≥ 7 days[B]	HCl	stable[H, WH]	stable[H]	stable[B]	2 yrs[HY]
Catecholamines	1 day[B]	bisulfate	≥ 7 days[B]	> 1 yr (pH 3)[W]	stable (pH < 3)[W]	2 yrs[HY]
Chlorides[2]	≥ 7 days[B]	acid (pH < 3)	≥ 6 mos[WH]	≥ 1 wk[H]	stable[B]	2 yrs[HY]
Creatine	4 hrs[B]				sev. mos[H]	2 yrs[HY]
Creatinine	4-7 days[H]	fluoride + thymol	3-4 days[C]	1-4 wks (PE)[H]	sev. mos[H]	2 yrs[HY]
17-Hydroxycorticosteroids		H_3BO_3	≥ 7 days[B]		stable[B]	
Magnesium[2]	≥ 7 days[B]	HCl	≥ 6 mos[WH]	(PS)	(PS)	(PS)
Manganese[2]	(PS)	HCl	≥ 6 mos[WH]	(PS)	(PS)	(PS)
Mucoproteins and related biocolloids						
Potassium[2]	≥ 7 days[B]	HCl	≥ 6 mos[WH]	sev. wks[C, 3]	(PS)	2 yrs[HY]
Serotonin (5-HIAA)	4-7 days[B]	H_3BO_3	1-4 wks[B]	1-4 mos (H_3BO_3)[B]	stable[B]	
Sodium[2]	2 wks[C]	HCl	≥ 6 mos[WH]	≥ 2 wks[C]	(PS)	2 yrs[HY]
Standard clinical analyses:[4]						
Albumin	≥ 4 days[B]				(PS)	
Ketone bodies[5]	unstable[H]			24 hrs[H]	(PS)	
pH	unstable[B]					
Sugar	4 hrs[B, 6]	CT[7]	5 days[H]	24 hrs[H]	stable[H]	
Sulfates	4 days[H]			≥ 4 days[H]	(PS)	(PS)
Total N		HCl (pH 4-5)	≥ 1 wk[B]		(PS)	(PS)
Zinc[2]	stable[B]	HCl	(PS)	stable[B]	stable[B]	(PS)

TABLE 3. - DURATION OF STABILITY OF URINARY CONSTITUENTS - Concluded

[1] Calcium tends to precipitate in the absence of acid. It is redissolved prior to analysis by addition of acid.

[2] Samples are best analyzed soon after collection to avoid precipitation of various constituents. If a precipitate is present it is redissolved prior to analysis by adding a small amount of acid.

[3] The sample is stable for several weeks provided there are no red cells present.

[4] Standard Clinical Analysis also includes: volume, color, microscopy, specific gravity, and turbidity.

[5] Acetoacetic acid is quite stable in some urines at room temperature but disappears rapidly in others, the disappearance appearing to be related to microbial action. In the presence of bacteria or yeast it may disappear completely in less than 24 hours, whereas if the solution is sterile, it is stable for 8-10 days. About 20% of the acetone present disappears in 24 hours at room temperature, but none is lost in the same period if the urine is kept in a closed container in the refrigerator. Ketone bodies may also disappear from urine *in vivo* in the presence of a urinary tract infection.

[6] The specimen is stable for 4 hours provided it is not heavily contaminated with bacteria.

[7] Cargille urinary preservative tablets buffer the urine to a pH of about 6. They are a mixture of benzoic acid, formaldehyde (produced from urotropin), and mercuric oxide.

and fluoride. These substances act primarily as antimicrobial agents. Toluene and chloroform are objectionable for in-flight use due to their volatility and noxious character. There are indications that formaldehyde may interfere with the determination of glucose in urine when employed in excessive quantities. Fluoride is a toxic agent, and is also objectionable because it tends to precipitate calcium from urine. Thymol, benzoic acid, boric acid, and mineral acids have achieved the widest acceptance in practice. Commercial urinary tablets such as Cargille and Urokeep are widely used clinically for preservation of urine specimens. Cargille tablets buffer the urine to a pH of about six and contain a mixture of benzoic acid, urotropin, and mercuric oxide. The composition of Urokeep tablets has not been specified.

Boric acid is effective in maintaining the stability of aldosterone, 17-hydroxycorticosteroids, and 5-hydroxyindoleacetic acid for periods of approximately one week. However, boric acid is ineffective in the preservation of catecholamines, for which stronger acid is required. An effective stabilizing agent is potassium bisulfate, used in amounts of 200 mg per ounce of urine (pH 3). This substance will preserve the urinary catecholamines for one to two weeks at 30°C. Urine acidified to pH 3 also preserves the activity of antidiuretic hormone for one week. To maintain the inorganic parameters in solution, mineral acids are customarily used. These acids preserve calcium, chlorides, potassium, sodium, zinc, and other inorganics for periods exceeding six months.

As indicated in the last three columns of Table 3, urine may be stabilized simply and effectively through reduced storage temperatures. Freezing will maintain the stability of virtually all parameters for periods of 90 days or more. Lyophilization appears to be effective for most parameters, with the notable exception of ketone bodies, which may undergo loss due to volatilization of acetone.

Serum and Plasma

Table 4 lists 35 parameters whose analysis is required from serum or plasma. In most instances the analyst has the option of selecting either specimen, but plasma is required for the following parameters: amino nitrogen, antihemophylic globulin, fibrinogen, fibrinolytic activity, plasma thromboplastic component, plasma volume (RISA 125), and prothrombin activity. An important consideration in handling serum and plasma is the avoidance of unnecessary exposure to sunlight, ultraviolet or even the usual lighting in a laboratory, since a 50% loss in unconjugated bilirubin may result within two hours.

In the absence of preservatives, a number of parameters deteriorate at room temperature within hours after the blood collection is made. The substances falling in this group are ACTH, amino nitrogen, antidiuretic hormone, antihemophylic globulin, creatine, fibrinolytic activity, and plasma thromboplastic component. The noted instability of antidiuretic hormone in plasma is in sharp contrast to urine, where the hormone has greatly enhanced stability. Table 4 presents specific data on the remaining 28 parameters in unprocessed serum and plasma. In general, it may be noted that untreated specimens are not acceptable for analysis beyond brief periods of storage, with the exception of the inorganic constituents.

As with other biological materials, serum and plasma tend to putrefy after collection with accompanying degradation of organic constituents. Fluoride and thymol are most commonly used as blood preservatives. Thymol acts as an antibacterial agent, while fluoride has inhibitory action on enzymes involved in glycolysis. Fluoride acts concomitantly as a weak antimicrobial agent and as an anticoagulant. The effectiveness of these agents is listed in Table 4. The stability of BUN is increased from one to five days, amino nitrogen from eight hours

TABLE 4. - DURATION OF STABILITY OF CONSTITUENTS OF SERUM OR PLASMA

Parameter	Method of preservation					
	Without preservative	Chemical		Refrigeration	Freezing	Lyophilization[1]
		Preservative	Duration			
Adrenocorticotrophic hormone (ACTH)	4 hrs (heparin)[I]				unstable (heparin)[I]	
Alkaline phosphatase	2 days[B]			1 wk[GO]	16 mos[H]	1 yr[HY]
Amino nitrogen (plasma)	8 hrs (Ox)[B]	Ox + NaF	5 days[B]	1 day[H]	stable[B]	
Antidiuretic hormone (ADH)[Z]	40 min (heparin)			1 hr (heparin)	≥ 17 days (heparin)[2]	
Antihemophylic globulin (AHG)[3] (plasma)	unstable[P]				≥ 1 yr[P, 5]	unstable[P, 4]
Bicarbonate	≥ 4 days[B]			(PS)	stable[B]	
Bilirubin (total)[6]	2 days[B]			4-7 days[H]	≥ 3 mos[B]	2-3 yrs[D, HY]
BUN	1 day[B]	F or F + thymol	5 days[H]	≥ 1 wk[W]	6 mos[B, W, WA]	2-3 yrs[D, HY]
Calcium	≥ 7 days[B]			≥ 1 day[W]	≥ 1 yr[W]	2-3 yrs[D, HY]
Chloride	≥ 7 days[B]			≥ 1 wk[H]	≥ 1 yr[W]	2-3 yrs[D, HY]
Creatine	4 hrs[B]	F or F + thymol	5 days[H]	24 hr[H]	sev mos[H]	
Creatinine	7 days[B]	F or F + thymol	5 days[H]	24 hr[H]	6 mos[WA]	2-3 yrs[D, HY]
Fat tolerance	4 days[B]				stable[B]	
Fibrinogen (plasma)	ca 7 days[H]			4 wks (EDTA)[H]	stable (CiB)[PR]	
Fibrinolytic activity (plasma)	unstable[SA]				stable (CiB)[PR]	

TABLE 4. - DURATION OF STABILITY OF CONSTITUENTS OF SERUM OR PLASMA - Continued

Parameter	Method of preservation					
	Without preservative	Chemical		Refrigeration	Freezing	Lyophilization[1]
		Preservative	Duration			
Immunoglobulins:				stable[HU]	stable[HU, R]	stable[G, HU, R]
γ - A Immunoglobulin	ca 4 days[B]			≥ 4 days[B]	stable[B]	
γ - G Immunoglobulin	ca 7 days[B]			≥ 7 days[B]	stable[B]	
γ - M Immunoglobulin	ca 7 days[B]			≥ 7 days[B]	stable[B]	
LDH Isozymes	ca 10 days[K]			unstable[B, K]	unstable[B, K]	2-3 yrs[HY, D]
Magnesium	≥ 7 days[B]			= 3 days[GO]	≥ 1 yr[W]	2-3 yrs[HY, D]
Manganese	≥ 7 days[B]				(PS)	(PS)
Mucoproteins and related biocolloids	2-7 days[H]				stable[B]	
NPN	1 day[B]	NaF	5 days[B]	≥ 1 wk[W]	stable[B]	2-3 yrs[HY, D]
PBI	≥ 5 wks[H]	Benzoic acid	15 days[KN]	4 wks[B]	3 mos[B]	2-3 yrs[HY, D]
Phosphates	4 days[B]			≥ 1 wk[H]	≥ 1 yr[W]	2-3 yrs[HY, D]
Plasma thromboplastic component (PTC) (plasma)	unstable[B]			unstable (Ca Ox)[B]	stable (CiB)[R]	
Plasma volume ($RISA^{125}$) (plasma)	≥ 60 days[7]			≥ 60 days[7]	≥ 60 days[7]	≥ 60 days[7]
Potassium	≥ 2 wks[H]			≥ 2 wks[H]	≥ 1 yr[W]	2-3 yrs[HY, D]
Prothrombin activity (plasma)	24 hrs[S, 8]			24 hrs (Ox + V)[B]	5 yrs[ST]	unstable[ST]
Protein (electrophoresis)	4 days[B]			1 month[H]	≥ 6 mos[B]	2 yrs[D]

TABLE 4. - DURATION OF STABILITY OF CONSTITUENTS OF SERUM OR PLASMA - Concluded

Parameter	Method of preservation					
	Without preservative	Chemical		Refrigeration	Freezing	Lyophilization[1]
		Preservative	Duration			
Sodium	≥ 2 wks[H]			≥ 2 wks[H]	≥ 1 yr[W]	2-3 yrs[HY, D]
Sulfates	4 days[B]			4 days[H]	stable[B]	(PS)
Thyroxine	≥ 11 days[H]			sev wks[B]	sev mos[B]	(PS)
Thyroxine binding prealbumin (TBPA)	≥ 2 wks[OP]			stable[OP]	stable[OP]	(PS)
Transferrins	4 days[B]			≥ 1 wk[H]	stable[HU, ST]	2 yrs[D]
Uric acid	3 days[B]	F or F + thymol	> 3 days[H]	3-5 days[H]	6 mos[B, WA]	2-3 yrs[HY, D]
Zinc	stable[B]			stable[B]	stable[B]	(PS)

Note: Use of the word plasma in the Parameter column indicates that its use is mandatory for the specified analysis.

[1] The data furnished by Hyland Laboratories and Dade Reagents refer to lyophilized normal sera stored at 2° - 8°C.

[2] The following special treatment is required: a) the plasma is divided into 1- to 2-ml aliquots and transferred to 13 x 160 mm test tubes into which are dropped pebbles of dry ice; b) to avoid loss of activity during the thawing process, CO_2 gas is bubbled through the sample.

[3] AHG is destroyed during clotting, hence it is not present in serum.

[4] There is some loss of AHG during the freeze-drying process.

[5] In order to successfully store AHG in plasma, the following factors must be kept in mind:
a) the specimen container must be sealed;
b) the storage temperature should be in the range of -30° or -70°C;
c) the blood samples must be obtained under conditions which do not impair AHG;
d) the plasma should be separated from the cells by adequate centrifugation and frozen rapidly.

[6] Sample must be stored in dark due to light sensitivity of bilirubin.

[7] This estimate is based on the half-life of I^{125}, which is 60 days. Precipitation of the plasma sample may occur in unfrozen specimens and this may lead to difficulty in procuring a sample for counting. This problem may be circumvented by pipetting out a known volume of plasma in-flight for post-flight counting.

[8] The blood specimens were obtained by means of venipuncture, using the Vacutainer method, taking care to be certain that the tubes were allowed to fill. The tubes were kept stoppered during centrifugation and unstoppered only at the time of sampling.

to five days, creatine from four hours to five days, and NPN from one day to five days. Chemical preservatives have not been found for the most unstable parameters, including ACTH, antidiuretic hormone and other substances which inherently possess periods of stability inadequate for the requirements of the manned space program.

Lyophilization has proven effective for preservation of many of the parameters listed in Table 4. Exceptions are plasma prothrombin activity and antihemophylic globulin, both of which are highly unstable. In a significant number of instances there is a dearth of information on the stability of the parameters in the lyophilized state. Further laboratory studies would appear warranted to fully determine the usefulness of lyophilization as a preservation technique.

Refrigeration of specimens at 5°C does not appear to be an acceptable method of long-term preservation for either serum or plasma. Under these conditions, parameters such as amino nitrogen, antidiuretic hormone, LDH isozymes, creatine, creatinine, plasma thromboplastic component, and prothrombin activity are stable for less than 24 hours. The other substances are stable for longer periods of time, but there is no marked improvement over storage at room temperature. With regard to proteins by electrophoresis, the table indicates that the serum specimen may be stored for a month under refrigeration. It should be noted that some investigators claim alterations occur in the α- and β-globulins despite refrigeration.

By far the most satisfactory technique for long-term preservation of serum and plasma is the use of freezing at temperatures below -10°C. At this temperature all parameters are stabilized for periods of two to three months or longer, with the exception of ACTH and LDH isozymes. Antidiuretic hormone is

stable for at least two to three weeks when frozen; the exact period of stability is not known, but may be substantially in excess of three weeks. Freezing of serum maintains the integrity of the proteins for long periods of time, although the samples must be rapidly thawed and thoroughly mixed before assay.

Whole Blood

Of the 17 tests designated for whole blood as shown in Table 5, 13 are hematologic while the remainder are biochemical. It is the hematologic tests which pose the greatest difficulty in stored blood specimens.

The biochemical parameters required in whole blood are hemoglobin, glucose, lactic acid, and methemoglobin. Hemoglobin is relatively stable and no difficulty is encountered in its preservation by refrigeration or freezing of blood. Glucose is unstable at room temperature and requires the use of fluoride for interim storage. For long-term storage the blood may be frozen to -79°C. Alternatively, blood is transferred to paper as a dry film. In this form both the glucose and hemoglobin contents are stable for extended periods of time. Both lactic acid and methemoglobin are unstable in untreated blood specimens, but methemoglobin may be maintained for three months or more in frozen blood. There is a lack of data on lactic acid stability in frozen blood, although lactic acid may be preserved as an extract by deproteinizing the blood sample with perchloric or trichloroacetic acid.

Standard procedures are available for determination of the hematologic parameters in fresh blood. Although blood may be prevented from clotting by use of a suitable anticoagulant, a serious problem arises in maintaining its viability for a protracted period. Blood consists of cellular and molecular components differing widely in function, complexity, and stability. Numerous studies

TABLE 5. - DURATION OF STABILITY OF WHOLE BLOOD

Parameter	Method of preservation						
	Without preservative	Chemical		Refrigeration	Freezing	Dried blood films	
		Preservative	Duration			On glass	On paper
Caryotyping	unstable			unstable	stable?[A, 1]		
Clot retraction	unstable[B]			unstable[B]	unstable[IO, 2]		
Clotting time	unstable[B]			unstable[B]	unstable[B]		
Glucose tolerance	1 hr[B]	NaF + thymol	≥ 7 days[B]	48 hrs (F)[W]	stable (-79°C)[HU, B]		18 mos[CO]
Hematocrit	2 days[B, 3]			4 days (Ox)[B, 3]	unstable[B, ST, HU]		
Hemoglobin	stable[H, 4]			stable[H, 4]	stable (-79°C)[HU, ST]		sev mos[RI]
Lactic acid	unstable[B]	PrA	≥ 7 days[B]	≥ 3 days (TCA)[H]			
Methemoglobin	4 hrs[B]			unstable[H]	stable[ST]		
Platelet adhesiveness	unstable[HE, 5]			unstable[HE, 6]	unstable[HU, ST]		
Platelet count	30 min[B, HE]			30 min (AnC)[B, ME]	unstable[M, HU, G, ST]	(PS)[7]	
RBC mass (sodium chromate^{51})	> 28 days[8]			> 28 days[3]	> 28 days[8]		
RBC survival (sodium chromate^{51})	> 28 days[8]			> 28 days[8]	> 28 days[8]		
RBC (total)	1 day[B]			unstable[B]	unstable[M, ST, G]		
Reticulocyte count	1 day[B]			1 day (AnC)[B]	unstable[HU, ST]	stable[HU, ST]	

TABLE 5. - DURATION OF STABILITY OF WHOLE BLOOD - Concluded

Parameter	Method of preservation						
	Without preservation	Chemical		Refrigeration	Freezing	Dried blood films	
		Preservative	Duration			On glass	On paper
WBC differential	1 day[B]			2 days (AnC)[B]	unstable[HU, ST]	stable[HU, ST]	
WBC motility and phagocytic activity	unstable			unstable	stable?[RW, HU, 9]		
WBC (total)	1 day[B]			2 days (AnC)[B]	unstable[HU, ST, A]		

Anticoagulants were used in these studies, with the exception of clotting time and clot retraction time.

[1] Whole blood was mixed with 30% glycerol and stored at -80°C after freezing under special conditions.

[2] Using moderate freezing rates, the clot-retraction activity (CRA) of platelets suspended in 50% plasma and 15% dimethylsulfoxide was retained with a 10-15% loss over the control CRA. Storage in liquid nitrogen for 38 days at -175°C resulted in a 34% reduction over the frozen, unstored control CRA.

[3] Mixture of dry ammonium and potassium oxalate.

[4] If tested by iron analysis of hemoglobin, samples should be stable indefinitely as long as the iron is in a form that can be uniformly dispersed so that there is no sampling error.

[5] Studies indicate the necessity for testing the platelet adhesiveness between 15 and 25 minutes after withdrawal of the blood.

[6] Citrated platelet-rich plasma.

[7] The technic of obtaining the platelet count by blood smears reveals semiquantitative data at best.

[8] The half-life of Cr^{51} is 27.8 days and is independent of temperature.

[9] The leukocytes are separated from whole blood, equilibrated with dimethylsulfoxide in a buffered salt solution, frozen carefully at a controlled rate, and stored in liquid nitrogen (-196°C).

have been conducted on the storage of blood for use in transfusion. These studies have clearly demonstrated that the blood cells and clotting system proteins are labile in storage. Maintenance of the viability of platelets is particularly difficult and may explain, in part, why determinations of clotting time and clot retraction time must be done with fresh blood. Iossifides et al. (1963) employed clot retracting activity as an in vitro test to determine the functional integrity of platelets during storage. Using a suspending medium consisting of 50% plasma in saline with 15% dimethyl sulfoxide (DMSO), the authors noted that storage in liquid nitrogen for 38 days resulted in a 50% reduction of clot retracting activity over the unfrozen control specimen.

It is generally agreed that freezing and storage at low temperature offers the most useful approach to the preservation of blood and its components for extended periods of time. Blood stored in this manner contains most biochemical parameters in an unchanged form. To maintain the integrity of the blood cells during the freeze-thaw cycle, chemical additives are necessary. Polge et al. (1949) discovered the protective action of glycerol in the freezing of spermatozoa. In 1950 Smith reported the protection of mammalian red cells by glycerol using a slow freeze-thaw technique. More recently, DMSO has been shown to be effective against freezing injury (Lovelock and Bishop, 1959). Rapid-freeze techniques have also been investigated using macromolecular additives, such as PVP and dextran (Bricka and Bessis, 1955; Rinfret).

Despite the demonstrated value of low temperature techniques for storing blood for transfusion purposes, there is some question concerning the applicability of cryogenic methods to the preservation of hematologic parameters. The

following considerations delimit the present state-of-the-art and provide insight into the problems involved in the storage of whole blood:

(1) Substantial destruction occurs in the cells during the freezing process, particularly the white blood cells and platelets.

The lability of the blood cells during the process of cooling to storage temperature is a matter of concern to specialists in the field. Dr. Rowe (1967) has made the following comments on this matter: "It is well documented in the literature that the lower the storage temperature the better the chance is for long-term preservation. The most convenient storage temperature is liquid nitrogen at -196°C, where no biological changes have been observed, as opposed to observable changes noted at higher degrees such as -80°C, or the temperature of dry ice, solid CO_2. Storage, then, does not seem to present any problem, but rather the problem lies in the method of getting down to the storage temperature and then getting back up from the lower storage temperature to ambient temperature."

(2) The consensus is that there is no single method which can be used to preserve the different cellular components in whole blood. The accepted practice (Rowe, 1967) is to isolate the leukocytes, platelets, and plasma from the red cells. Each component is then stored separately by an appropriate technique. Dr. Rowe has successfully preserved leukocytes by freezing them in a cryoprotective additive of 10% dimethyl sulfoxide or glycerol using a slow freezing rate, of the order of 1°C per minute. This technique also seems to work well with platelets, although platelets are quite fragile and are considerably more difficult to recover in good yield. Of all the freezing procedures available, Dr. Rowe believes that the rapid freeze procedure employing low glycerol (14%) and liquid nitrogen gives the highest yield of intact erythrocytes. With this method he obtains

94% overall recovery of erythrocytes with minimum damage to the cells. The recovered cells are indistinguishable from cells in fresh blood, on the basis of RBC survival studies. The results of these studies are in the process of being prepared for publication. Dr. Rowe has also recently developed a convenient method for freezing droplets of blood. Although this technique is not suitable for transfusion purposes, it is very useful for blood grouping.

(3) There is a paucity of data in the literature pertaining to the stability of the hematologic parameters in whole blood listed in Table 1; most stability studies are concerned with the recovery of cells for transfusion purposes.

It is the consensus that whole blood specimens stored in the frozen state cannot be employed for the following tests: hematocrit, caryotyping, platelet adhesiveness, platelet count, RBC total, reticulocyte count, WBC differential, WBC motility and phagocytic activity, and WBC total. There are presently no known storage techniques for whole blood to accommodate either of these parameters, or the clotting time and clot retraction time tests. However, it may be possible to stabilize several of these parameters by the following techniques:

(1) Smears may be prepared directly after the blood specimen has been drawn for a reticulocyte count and for a WBC differential. These smears are probably stable for long periods. A blood smear may also be prepared in flight for a platelet count, with the limitation that the assay is at best semi-quantitative.

(2) Some limited success in stabilizing the caryotyping parameter has been obtained by Atkins (1962). He used glycerolized blood stored at -80°C as the source of the lymphocytes for his culture studies. Dr. Rowe (1967) believes that the following parameters may be preserved by the application of slow freezing

storage techniques to isolated leukocytes: cytogenic studies of leukocytes, WBC motility and phagocytic activity, WBC differential, and WBC total count.

(3) Reticulocyte count presents no problem upon recovery of red cells; in fact, they appear to survive better than older cells (Rowe, 1967). With his low glycerol-liquid nitrogen freezing process, Dr. Rowe obtains a high recovery of erythrocytes with minimum change in RBC survival and RBC mass properties. However, the platelet adhesiveness and platelet count parameters are more difficult to preserve and satisfactory procedures for doing so have yet to be developed.

(4) Dr. Myhre (1967) believes that it may be possible to stabilize the total RBC count, total RBC mass, and RBC survival parameters in whole blood for at least six months. He recommends refrigeration at 4°C in the presence of Alsever's solution or a special ACD preservative.

The standard test for RBC mass employs hexavalent radiochromium-51, supplied as sodium chromate, to tag the cells in citrated blood obtained from the donor. A portion of this tagged blood is then returned intravenously to the donor, and a blood sample withdrawn 30 minutes later. Since the half-life of chromium-51 is 28 days, and only 30 microcuries of chromium are used in the test, it is likely that the blood specimens could not be counted reliably beyond a 30 or 40 day period. However, tagging the blood with 90 or 120 microcuries of chromium might allow storage of the blood specimens for two or three months. Incidentally, it should be noted that the test for RBC mass requires a determination of the hematocrit value. In view of the unacceptability of stored blood for an hematocrit determination, as indicated above, it would appear necessary to obtain the hematocrit in flight by use of a spaceborne centrifuge.

The test for RBC survival also employs radiochromium-51 for tagging of

the cells. The tagging may be done either in vitro or in vivo. In either instance the hematocrit must be obtained. RBC survival studies can be performed by an in vitro tagging using 150 microcuries to follow the radioactivity for as long as 110 days. If injected intravenously, 200 microcuries of chromium-51 yields a red blood cell activity which persists for approximately 35 days. Following the injection of 300 microcuries, the radioactivity in red blood cells can be followed with a scintillation well type counter for up to 45 days. The radioactivity in the cells persists at a significant concentration for up to 60 days in the normal. Cline et al. (1962) have employed tritiated diisopropyl-fluorophosphate (DFP) for measuring red cell survival in humans. The long physical half-life of tritium (12.3 years) is very useful when prolonged storage periods of the labeled red blood cells or the radioactive labeling reagent are necessary. The stability of tritiated DFP is such that sealed ampules can be used up to one year after preparation.

Sweat and Feces

Sodium, potassium, calcium, and chloride are designated as the only required parameters in sweat and feces. These inorganic substances are highly stable, hence the specimen may be stored without preservative at room temperature. Two precautions must be taken in handling sweat, however. Immediately upon collection, the specimens must be placed in small, tight containers in order to minimize loss of volume due to evaporation. If preservatives are not added, there is every likelihood of bacterial contamination. Bacterial growth may lead to incorporation of potassium ions within the bacterial cell, resulting in non-homogeneity of the sweat sample. It is therefore necessary that the entire sweat sample be employed for analysis.

One consideration in the storage of sweat and fecal specimens in the formation of objectionable odors due to breakdown of organic compounds resulting from bacterial action. The use of lyophilization or freezing techniques may be considered in an effort to reduce this problem. As noted in Tables 6 and 7, virtually any form of storage appears to be acceptable for sweat and fecal specimens. If the lyophilization technique is selected, it is a good precaution to neutralize the sweat specimens to avoid possible loss of chloride.

Microbiological Flora

The microbiological source material has not been specified by NASA. For the purpose of this study, attention has been directed to bacterial and viral specimens which may be obtained from nose, mouth, skin and throat.

Two methods are available for the preservation of microbiological specimens. The first is based on freezing at reduced temperatures. A temperature in the range of -60°C is required for the preservation of most viruses. Bacteria are more labile than viruses and require more stringent conditions for their preservation. In 1966, Rinfred et al. developed a quick-freezing and thawing process for preserving bacterial suspensions. In this process, the chilled sample is sprayed onto a linearly moving film of liquid nitrogen, and the frozen droplets sieved and stored in a liquid nitrogen refrigerator at -196°C. During the thaw cycle, the frozen material is fed gradually into a rotating aluminum pan, kept at 47°C, containing 7% polyvinyl-pyrrolidinone (PVP) in an isotonic saline solution. Depending on the freezing equipment, the survival rates were found to be 20 to 61% for Azotobacter, 95 to 122% for E. coli., 59 to 102% for Staph. aureus, 12 to 23% for Aspergillus niger, and 34 to 52% for yeast.

Freeze-drying represents the second technique for preserving viruses and bacteria. Freeze-drying of micro-organisms is generally accomplished for long term preservation at ambient temperatures, and it is important to appreciate that the conditions necessary for survival following drying are not necessarily those for optimum preservation or subsequent storage. The most important factor determining survival rates appears to be the medium in which the micro-organisms are dried (Meryman, 1966). Greaves, 1960, found that some cultures dried in mist. desiccans (consisting of 75% serum, 25% broth with 7.5 g. of glucose per 100 ml. of medium), remained viable for as long as ten years. Greaves (1964) specifies the following requirements for a suitable drying medium:

(1) The preparation must be prevented from falling below 1% residual moisture. This is achieved by adding sugar in a concentration of 5 to 10%.

(2) The addition of sodium glutamate to prevent the carbonyl groups in the medium from reacting with bacterial proteins.

(3) The addition of 5 to 10% PVP or dextran to give body to the freeze-dried preparation.

The initial freezing step prior to drying, is critical. Very rapid freezing, with the formation of intracellular ice crystals, is known to damage certain organisms. Greaves found, however, that rapid-freeze injury at -13°C could be minimized by the addition of glucose, PVP, or both, to the drying medium. Muggleton (1964) reports a marked increase in the survival of micro-organisms when the drying temperature was lowered to -35°C. In this study approximately 60% survival was obtained with Staph. aureus, E. coli, Streptomyces griseus and Penicillium notatum. Scott (1960) shows the need to maintain a 1% residual moisture content, and to exclude oxygen during the storage period following the freeze-dry process.

Miscellaneous Storage Methods

As noted in the discussion above, chemical preservatives, refrigeration, freezing, and lyophilization constitute the principal techniques employed for the preservation of biological specimens. In this section some attention is given to absorption and ion exchange techniques, which have received limited application for specimen storage.

Absorption. - The storage of measured quantities of whole blood or serum on paper for subsequent analysis has been successfully applied to the following parameters: hemoglobin, alkaline phosphatase, glucose, and phenylaline. Rice (1967) has shown that when 20 μl samples of whole blood are applied to Talliqvist test paper, they may be stored for many months at room temperature without altering the hemoglobin content. Comstock et al. (1966) have developed a method for the collection and preservation of blood specimens for glucose determinations by drying aliquots of blood on a paper carrier. Exactly 20 μl of finger blood are collected in a micro pipet and transferred quantitatively to a circled area on the filter paper sample carrier. Multiple samples can be collected on a single specimen carrier. In this way a glucose tolerance test can be performed on specimens obtained by finger puncture and applied in sequence along the paper, with no opportunity for the specimen sequence to be altered. A procedure for fixation of protein on the paper carrier is described which allows glucose to be eluted with water. The results are accurate and reproducible and equivalent to those obtained by conventional, routine methods for glucose (Somogyi-Nelson). The stability of specimens stored in this manner is more than adequate for in-flight use. Stability studies show that glucose values remain unchanged for at least one

TABLE 6. - DURATION OF STABILITY OF THE CONSTITUENTS OF SWEAT

Parameters	Method of preservation			
	Without preservative[1]	Refrigeration	Freezing	Lyophilization[2]
Calcium	stable[GD]	stable[GD]	stable[GD]	(PS)
Chlorides	stable[GD]	stable[GD]	stable[GD, LI]	(PS)
Potassium	stable[GD]	stable[GD]	stable[GD]	(PS)
Sodium	stable[GD]	stable[GD]	stable[GD]	(PS)

[1] Microbial growth occurs in the absence of preservative, with attendant loss of potassium due to its incorporation within the bacterial cell. The entire sample must therefore be used for analysis.

[2] The sample should be made neutral or alkaline prior to lyophilization to avoid loss of chloride as HCl.

TABLE 7. - DURATION OF STABILITY OF THE CONSTITUENTS OF FECES

Parameters	Method of preservation			
	Without preservative	Refrigeration	Freezing	Lyophilization
Calcium	≥ 7 days[B]	stable[B]	stable[B]	(PS)
Chlorides		(PS)	(PS)	(PS)
Potassium	stable[B]	stable[B]	stable[B]	(PS)
Sodium	stable[B]	stable[B]	stable[B]	(PS)

year when stored at room temperature without desiccation and for more than 18 months when stored in a desiccator at room temperature. The authors (Comstock, 1966) project that other soluble nonprotein molecules which are more stable than glucose and ordinarily present in blood can also be stored for prolonged periods by rapid drying of blood specimens on paper.

Procedures are presently available for storing blood on paper for enzyme analysis, e.g., cholinesterase activity (Augustinsson et al., 1953). With this technique, the cells do not need to be separated from the plasma. The blood is absorbed on filter paper and air dried. The samples remain unchanged for one week when stored at room temperature and several weeks when stored in the refrigerator. Bourdillon et al. (1966) are experimenting with methods for autoclaving blood spots on filter paper for urea, uric acid, and phenylalanine analyses.

A multiple sample analyzer has been patented by Warner-Lambert Pharmaceutical Co. (1966), which is suitable for automation. Measured samples are applied to a tape and are subsequently treated with the appropriate reagents as they are carried along to different stations by the moving tape. Using conventional detectors in conjunction with the moving tape, determinations for ketone bodies, bile pigments, hemoglobin, adrenaline, peptides, and nucleotides have been made in urine and blood specimens. Stability studies of these parameters on the tape remain to be carried out.

The storage of small, measured volumes of blood or serum on kieselguhr has been found suitable (Simmonds, 1960) for the conservation of cholinesterase,

alkaline phosphatase, and urea. All the procedures involved are simple and can be adapted to routine use in laboratories under terrestrial conditions. Stability studies of blood adsorbed onto tablets of kieselguhr yield the following data:

(1) Blood stored at 4°C on kieselguhr retains 85 to 90% of its original cholinesterase activity for a week and approximately 80% for two months or more. Samples kept at 20°C retain about 80% of their activity for approximately two weeks.

(2) Alkaline phosphatase in serum is stable on kieselguhr for six weeks or longer if kept at -15°C. At 4°C the activity is retained fairly well for up to three weeks, but at 20°C the enzyme rapidly deteriorates.

(3) Blood samples stored on kieselguhr tablets for urea assay are stable for at least two months when kept either at 4°C or 20°C.

Ion exchange techniques. - The preservation of human blood as an unadulterated fluid has long been the goal of blood banking techniques. Emphasis in this direction was given by the work of Steinberg (1944) and others who described the use of a cation exchange resin to decalcify blood and render it incoagulable. These studies indicated that resin-treated blood is satisfactory for hematologic, serological, and biochemical examinations. Platelets retain their ability of inducing clot retraction for approximately one to two days, and thromboplastic activity for a much longer period of time.

A convenient "Blood-Pack" unit is available from Fenwal Laboratories which employs Dowex 50 resin on the sodium cycle for routine blood collections.

According to Walter (1950), blood decalcified by this technique has a clotting mechanism which is functionally unaltered. The RBC, WBC, and platelet counts are unchanged. Thromboplastic activity is retained. The hemoglobin content is unchanged, but the calcium, magnesium, and potassium levels are strongly depressed. Work by Emerson (1950) suggests that blood decalcified by contact with a resin is comparable in viability with ACD blood during at least ten days of storage. In general, however, blood prepared in this manner and then refrigerated at 1 to 5°C is not stable for more than five to ten days with respect to the parameters in Table 1.

Ion exchange resins are widely used as analytical tools in biochemistry. They are employed for resolution of complex mixtures; for cation or anion replacements; and for isolation of discrete compounds from complex mixtures. In theory it should be possible to use ion exchange resins for storage of charged molecules. On this basis, a number of parameters in biological specimens might be immobilized by exposure to resins. Very little work has been done along these lines, however, and it remains to be determined whether resins offer sufficient promise to warrant detailed research studies.

Special Problems in Handling Blood Specimens

Improper collection or handling of biological specimens prior to storage can complicate or even invalidate the results of subsequent analyses. In this connection there are a number of special biological problems which are considered here.

Separation of serum and plasma from blood. - In preparing serum and plasma it is important that the sample be separated from the blood cells to prevent exchange of electrolytes between cells and extracellular fluid. The preferred

procedure is to remove the fluid within one to two hours of collection. This is especially important for potassium, but if the separation is delayed, the blood is best kept at room temperature. It has been noted by Goodman et al. that a much greater increase occurs in serum potassium levels in clotted specimens stored at 4°C than at 25°C. Plasma collected in tubes containing large concentrations of heparin (200 to 300 units per 5 ml blood) can be left in contact with blood cells for more than four hours without any effect on the potassium concentration.

The failure to separate plasma promptly from blood cells may cause changes in several of the parameters listed in Table 1. One example is uric acid, which undergoes uricolysis when kept in prolonged contact with erythrocytes. Serum uric acid is more stable while standing on the clot due to the decreased contact between serum and cells.

Hemolysis. - When either serum or plasma is required for analysis, hemolysis must be avoided in obtaining the blood specimen. This is done by using a dry syringe and needle, and slowly ejecting the blood from the syringe after removing the needle. In preparing serum, the freshly drawn blood is transferred to a tube without anticoagulant and permitted to clot spontaneously. When this is done under terrestrial conditions, the clot is gently ringed with a wooden applicator stick or thin rod prior to centrifuging down the clot. Excessive ringing produces undesired hemolysis. Allowing the clot to retract at room temperature before centrifugation is desirable, since there is less likelihood of hemolysis and a larger volume of serum is obtained.

Hemolysis must be avoided because it interferes with a number of serum and plasma analyses. Almost all serum enzymes are present in much higher concentrations in the erythrocytes than in plasma. Any visible amount of hemolysis

will usually produce elevated serum or plasma enzyme levels. In view of the high concentration of magnesium and potassium in the erythrocytes as compared to plasma, specimens showing even mild hemolysis cannot be used for the analysis of these constituents. If an unmodified Malloy-Evelyn diazo method is used for determination of serum bilirubin, then any degree of hemolysis interferes in the azo-coupling reaction and results in decreased levels. It may also be noted that hemolysis affects the analysis of total protein by the biuret reaction. However, this interference may be overcome by processing a serum blank. Other analyses that are influenced (elevated) by hemolysis are NPN, lactic acid and pyruvic acid.

Most chemical analyses are carried out on serum rather than plasma, but plasma is required for a substantial number of tests. In preparing plasma it is necessary to avoid hemolysis by use of the proper anticoagulant. Sodium fluoride is an effective anticoagulant but is employed in relatively high concentration. Under these conditions it is likely to produce hemolysis as well as significant shifts in water from the blood cells to plasma. Hemolysis may also occur due to improper use of oxalate as an anticoagulant.

Anticoagulants. - Chemical reagents that prevent coagulation are routinely used when whole blood or plasma is required for analysis. Anticoagulants owe their action to inhibition of conversion of prothrombin to thrombin by the removal of calcium ions (fluoride, oxalate, citrate, EDTA) or by other mechanisms (heparin). A prime consideration in the choice of an anticoagulant is that it not interfere with the analyses which are to be conducted. As noted above, fluoride causes hemolysis when employed in high concentrations which may contraindicate its use, although it may be acceptable in lower concentrations (John, 1926). The use of fluoride is contraindicated when plasma enzymes whose activity is inhibited

by fluoride ions are to be determined. EDTA is a powerful metal chelating agent and must be regarded as a potential interferent in cation analysis. Lactic dehydrogenase is reported to lose activity in oxalated plasma.

Plasma containing calcium-removing anticoagulants (oxalate, citrate, EDTA) cannot be used for calcium determinations, and plasma containing sodium or potassium salts of anticoagulants is unsuited for sodium or potassium determinations. Heparin at a concentration of 0.5 mg/10 ml contains a small amount of sodium or calcium as the salt, and this may alter results. If a urease technique is used for determining urea nitrogen, the sample must not be collected in a tube containing an enzyme inhibitor or anticoagulants containing ammonium salts. However, plasma or whole blood collected with these anticoagulants may be assayed for urea nitrogen by direct chemical methods, such as the diacetyl monoxime procedure.

A serious objection to the use of oxalates as anticoagulants lies in the alteration of concentrations of plasma components. The hematocrit using potassium oxalate may be 10% less than that obtained with heparin. This reduced erythrocyte volume results from a water shift from the erythrocytes to the plasma caused by the addition of the salt to the plasma phase. A 10% decrease in hematocrit results in a dilution error of plasma constituents of 5%. Substantial water shifts are also caused by sodium citrate as well as by sodium fluoride when employed in relatively high concentrations. Sodium polyanetholsulfonate, commonly called Liquoid, is a good anticoagulant which has no effect on erythrocyte volume. However, it has been reported to interfere with the determination of protein.

Specimen Freezing and Storage Unit

An analysis of the duration of stability tables reveals that freezing of the specimens will be required to insure preservation of most of the parameters. In view of this, some estimate of the volume, weight and power requirements of the specimen freezer is appropriate.

Inadequate information exists at this time to properly define the quantity of specimens which may have to be stored for a given mission. Table 2 indicates that in order to perform all the tests specified in Table 1, more than 20 ml of serum is required. This, in turn, requires 50 ml of whole blood - a quantity which must be obtained by venous puncture. This would undoubtedly be psychologically unacceptable if obtained on a daily basis, so, for purposes of this analysis, a weekly interval was selected. Also, sweat and feces were included in the freezer although freezing is not strictly necessary for constituent stability.

It was assumed that 20 ml of serum; 36 ml of urine; 7 ml of blood; 4 grams of feces; and 4 ml of sweat constituted a sample set. Because it is common clinical laboratory practice to perform the tests in duplicate, the specimen sizes were doubled for a second evaluation.

Twelve hours are anticipated for lowering the temperature of the specimens from 37°C to -80°C. The specimens would be at refrigeration temperatures within two hours, which insures adequate stability of the parameters for the remaining ten hours.

A system utilizing liquid oxygen as the refrigerant is envisioned. The oxygen would be boiled off with the heat of vaporization providing the necessary cooling. The oxygen vapor could be vented to the cabin or overboard if desired.

Pressure to circulate the oxygen could be obtained from the cabin environmental oxygen supply or by hand pumping.

Because of the weightless environment, convection currents will not be established within the freezer. Therefore, the unit should be designed to allow storage of each specimen adjacent to a refrigerated wall.

If a 90 day mission is assumed and 12 sets of specimens are obtained, the freezer with its liquid oxygen supply would occupy 300 cubic inches, weigh 15 pounds and consume no power. If the specimens are obtained for duplicate analysis, 500 cubic inches and 22 pounds would be required.

The freezer box would occupy 65 cubic inches for single samples and 125 cubic inches for singles and duplicates. For return to earth, the cryogenic supply is disconnected. The insulated box will retain the specimens in a frozen state for more than eight hours. This is sufficient time for re-entry and recovery, after which a cryogenic supply could be attached and the specimens transported to the laboratory.

TRADEOFF ANALYSIS AND RECOMMENDATIONS

In any recommendation of methods for preservation of biological specimens, the required storage time must be specified. As these times are not completely defined at present, a typical mission time is assumed for purposes of this discussion.

The storage times presented in the previous section have been quantized into typical mission durations and are presented in Figures 1 through 5. It is presumed that experiments requiring specimen storage will not be scheduled for mission times of less than 14 days. Therefore, the entries for less than 14 days

in the charts include totally unstable parameters. Where previous tables listed parameters as "stable" or "probably stable," they have been shown as stable for greater than 180 days. Wherever information is known on the stability of a parameter for a given preservation technique, an entry has been made. Except where previously noted, no times have been extrapolated beyond known stable periods.

It can be seen from Figures 1 through 5 that the most critical specimens from the standpoint of stability are whole blood, serum and plasma. Very few of the parameters specified for these specimens are stable for greater than 14 days without freezing. In whole blood, the hemotologic parameters are difficult to store by any method. Even by using a combination of freezing and dried films, only half of the parameters in whole blood can be expected to remain stable for typical mission durations.

Figure 6 presents an equipment evaluation summary of the various methods of preservation. The rankings are on a one to four basis with four signifying equipment having minimum volume, weight and power requirements, minimum complexity, maximum zero gravity compatibility and maximum stability time. On the other end of the scale, the rating of one signifies maximum volume, weight and power, etc. It can be seen from this tabulation that storage without any preservation is the easiest to mechanize but results in unacceptably short stability times for most parameters. Chemical preservation is slightly more complex but allows a longer storage period for some parameters.

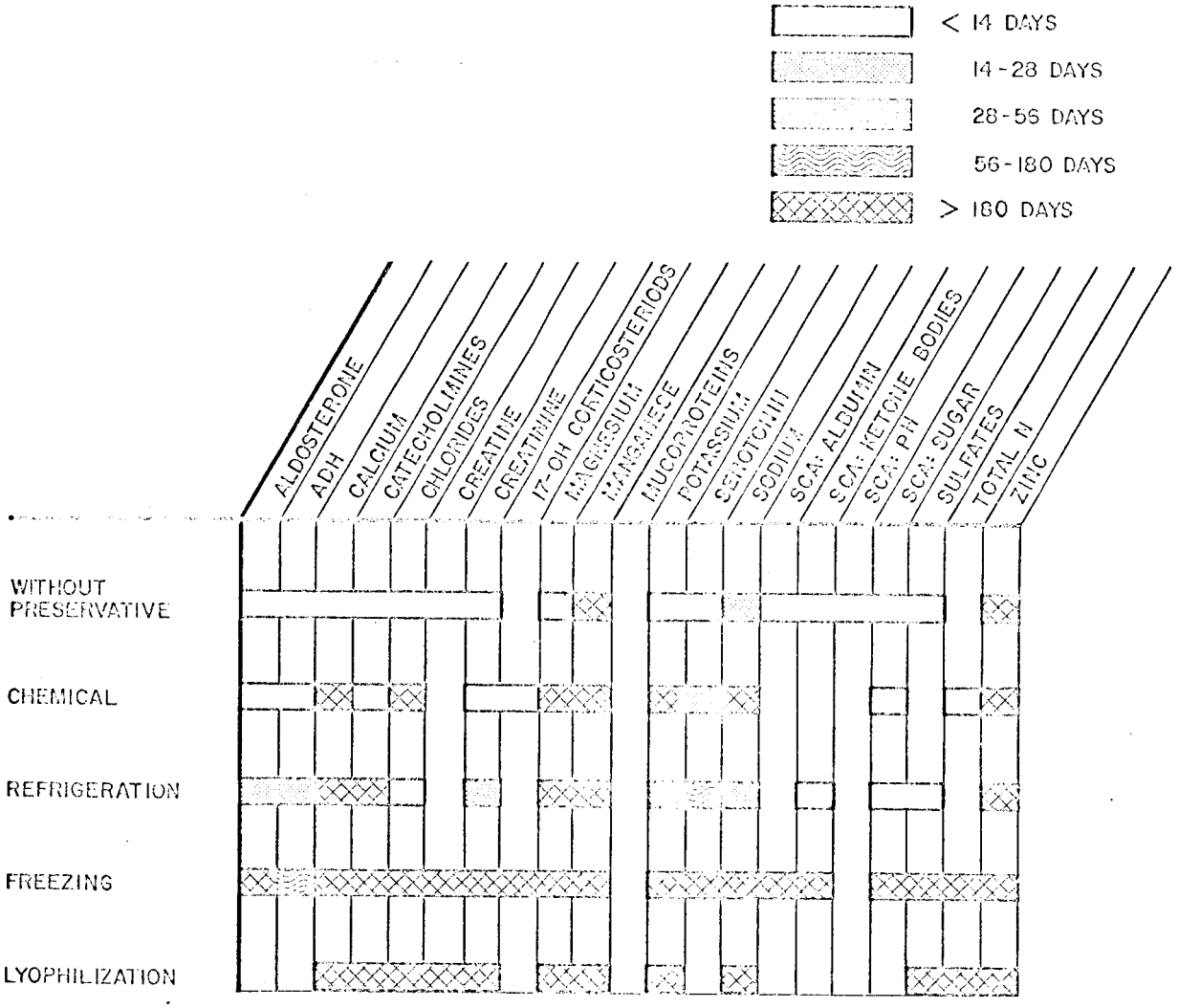
Refrigeration offers little advantage over freezing, particularly if cryogenics are available. If power is required from the spacecraft, refrigeration

may offer a small savings in this area. However, the greatly increased storage time available through freezing heavily weighs the trade-off in favor of this technique.

Lyophilization is the most complex of the methods and offers an advantage over freezing in only one instance: LDH isozymes in serum or plasma. The requirement for this parameter should be carefully assessed and alternative methods of obtaining the measurement investigated before establishing the requirement for a lyophilizer.

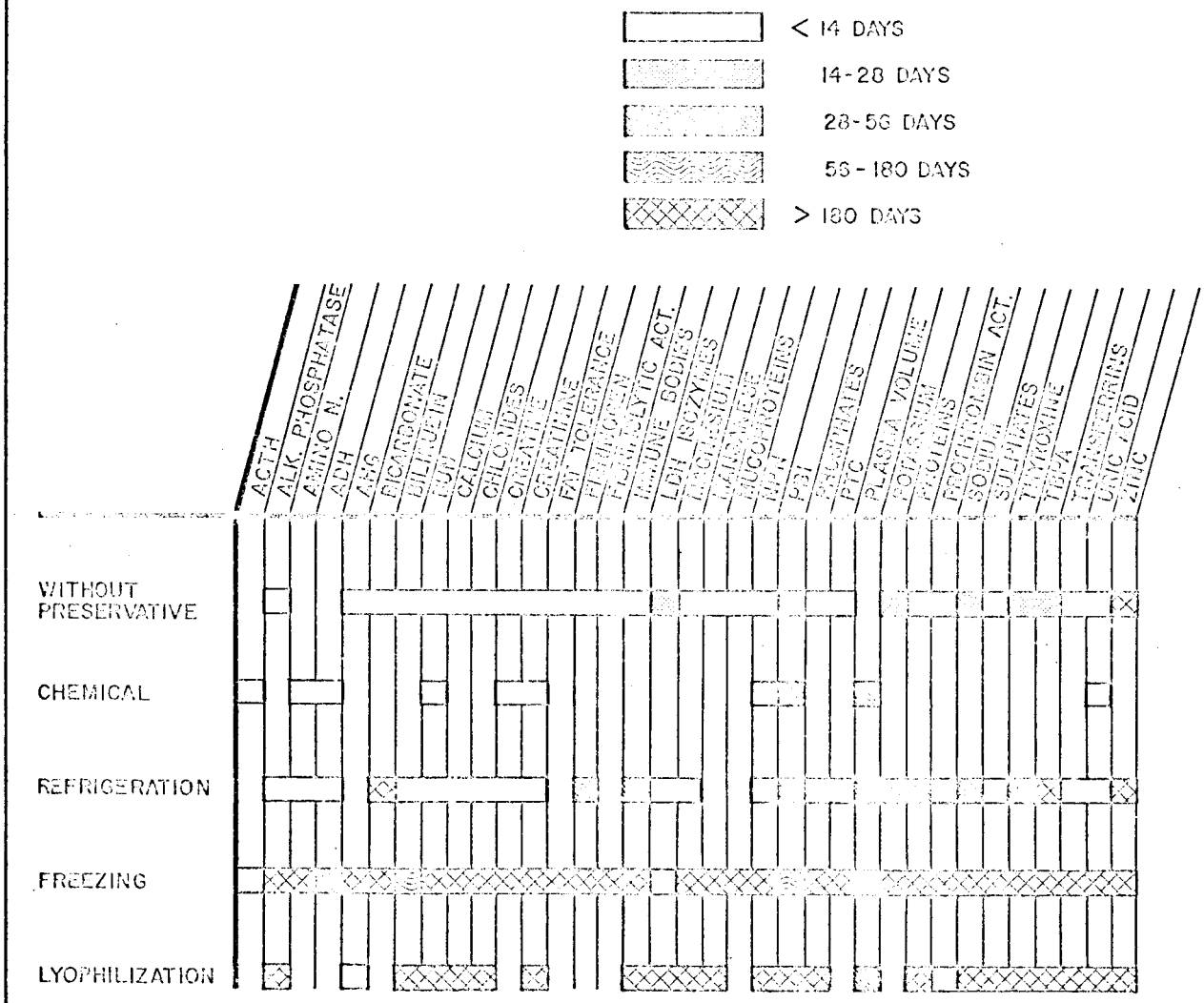
It is recommended, therefore, that development of a space-qualified specimen freezer and storage unit be undertaken for the preservation of whole blood, urine, serum and plasma. Additionally, provision should be made for obtaining dried blood smears on paper or glass for platelet count, reticulocyte count, and WBC differential. While the method of preservation for sweat and feces is relatively unimportant for the parameters specified, the freezer could be used for these specimens with little penalty in size, weight and power over alternate storage methods.

The vo The volume, weight and power requirement for the specimen freezer have been presented in the previous section. For volume, 300 to 500 cubic inches were estimated and 15 to 22 pounds for weight. No power from the spacecraft would be required.



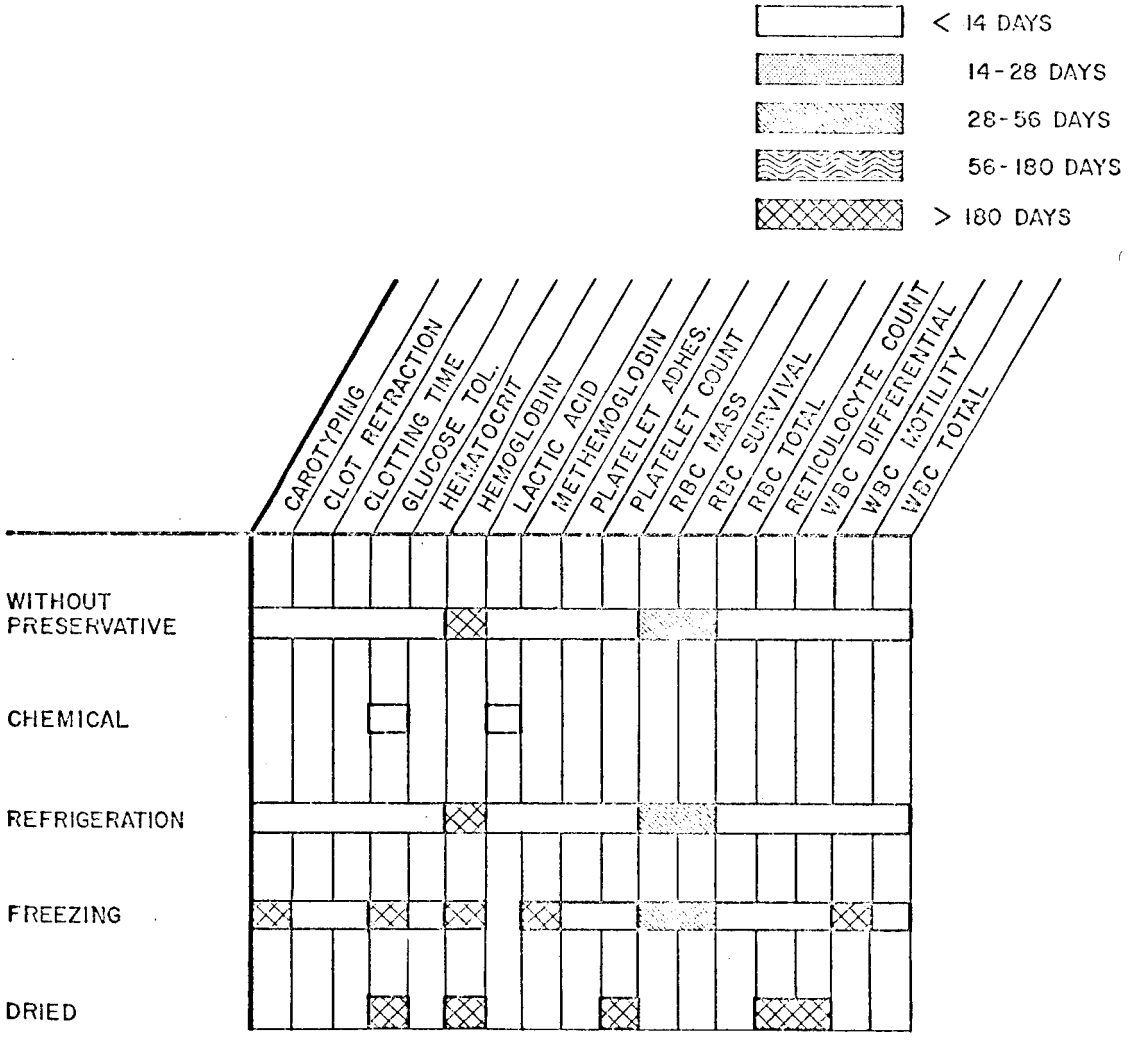
STABILITY OF THE CONSTITUENTS OF URINE

FIGURE 1



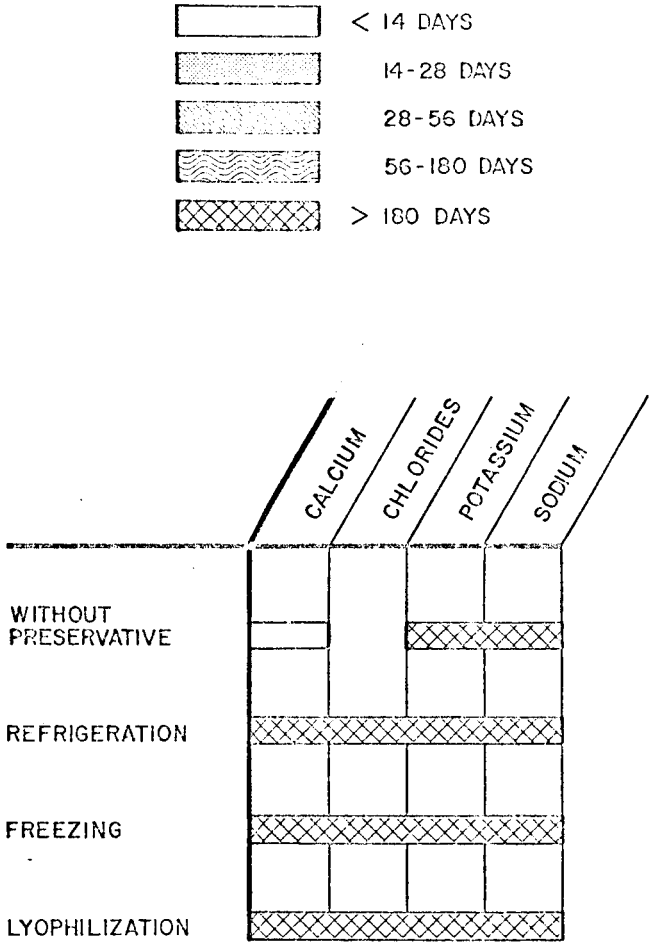
STABILITY OF THE CONSTITUENTS OF SERUM AND PLASMA

FIGURE 2



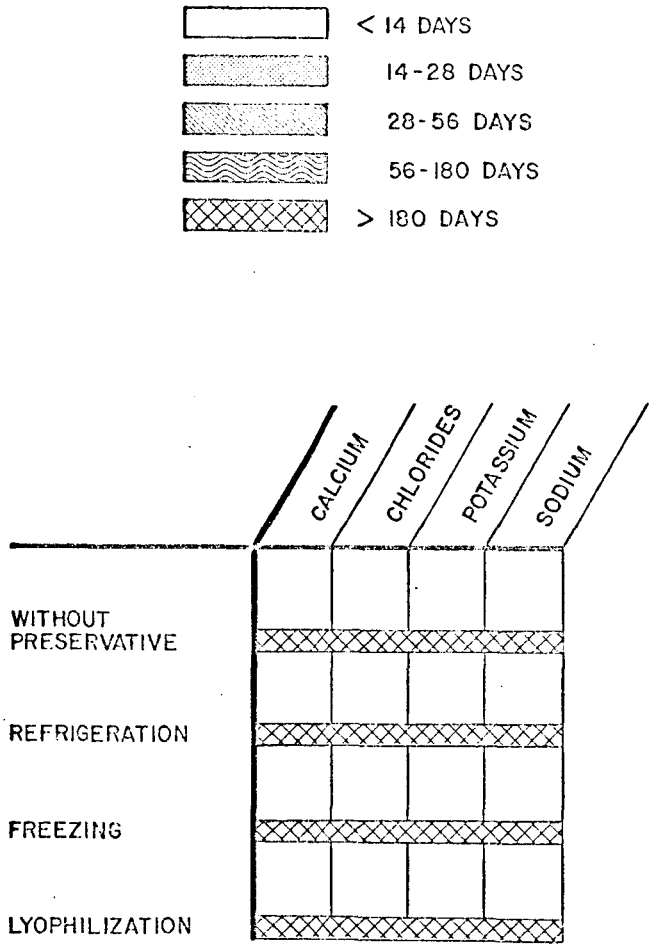
STABILITY OF THE CONSTITUENTS OF WHOLE BLOOD

FIGURE 3



STABILITY OF THE CONSTITUENTS OF FECES

FIGURE 4



STABILITY OF THE CONSTITUENTS OF SWEAT

FIGURE 5

	EQUIPMENT VOLUME	EQUIPMENT WEIGHT	EQUIPMENT POWER	OPERATIONAL COMPLEXITY	ZERO GRAVITY COMPATIBILITY	STABILITY TIME
WITHOUT PRESERVATIVE	4	4	4	4	4	1
CHEMICAL	3	3	4	2	3	2
REFRIGERATION	2	2	2	3	2	3
FREEZING	2	2	2	3	2	4
LYOPHILIZATION	1	1	1	1	1	4

EQUIPMENT EVALUATION SUMMARY

FIGURE 6

APPENDIX A

Notes and References Applicable to Tables 3 Through 7

Notes

1. "Stable" signifies stability for an extended period of time, exceeding two to three months.

2. Arabic numerals represent footnotes.

3. Refrigeration signifies a temperature of about 5°C.

4. Freezing indicates a temperature lower than -10°C.

5. The data in the "Without Preservative" and "Preservative" columns refers to storage at room temperature (20 to 30°C).

Abbreviations

AnC = Anticoagulant

Ca Ox = Calcium oxalate

CiB = Citrate buffer

CT = One Cargille tablet per ounce urine

EDTA = Disodium ethylenediaminetetraacetate

F = Fluoride

H_3BO_3 = Boric acid

NaF = Sodium fluoride

Ox = Oxalate

PE = Petroleum ether

(PS) = Probably stable

PrA = Perchloric acid filtrate

TCA = Trichloroacetic acid filtrate

V = Veronal buffer

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